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NUTRITION REVIEWS

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NUTRITION REVIEWS

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ANOMALIES IN THE PREDICTION OF NUTRITIONAL DISEASE

Nutritional science has made such progress within the last generation that it is possible partly to predict the stigmata likely to occur in a population group from its dietary pattern. This is true for the classical deficiency diseases—xerophthalmia, beriberi, pellagra, and, to a degree, scurvy. If, in addition to dietary pattern, the habitual degree of exposure to available ultraviolet radiation is known, it is possible to forecast the occurrence and severity of rickets and osteomalacia. If both diet and incidence and intensity of parasitism (malaria, ancylostomiasis, schistosomiasis, and the like) are known, the approximate occurrence and severity of iron deficiency anemia are predictable.

Nevertheless, there are a number of situations in which anomalies prevail. This paper refers to two types of anomalies: group I includes diseases which are strongly associated with or modified by nutrition but are less common in certain contexts than would be expected, and group II includes diseases or conditions, at one time firmly held to be primarily of nutritional origin, but now regarded as of uncertain or unknown etiology. To illustrate the first group there is the rarity of infantile scurvy in urban Bantu prematurely weaned infants, and much lower incidence of "alcoholic" cirrhosis and myocardial infarction in the South African Bantu, than would be expected. In the second group there is "nutritional" or cryptogenic heart disease and gynecomastia in the Bantu, and vesical calculi in certain Indian populations.

GROUP I

Infantile Scurvy in the Bantu

In 1955 there were 46 admissions to the Hospital for Sick Children in Toronto for infantile scurvy (W. S. Whelen *et al.*, *Canad. Med. Assn. J.* **78,** *177 (1958)*). In Johannesburg, at Baragwanath Non-European Hospital (1750 beds), during the past ten years there have been only four proven cases of infantile scurvy; in other words, among prematurely weaned Bantu infants the disease is virtually nonexistent. In harmony with this observation is the finding of satisfactory levels of plasma ascorbic acid, even in babies with kwashiorkor, values consistent with an intake of several milligrams of the vitamin daily. Nevertheless, careful examination of total diets of large numbers of such infants for ascorbic acid has revealed zero concentration in a large proportion of cases. Intensive interrogation excludes the possibility that *all* weaned Bantu infants are receiving sources of ascorbic acid unknown to their mothers, Bantu social workers, and us. We must, therefore, conclude that under certain specific conditions, a measure of intestinal biosynthesis takes place (M. Andersson *et al.*, *Brit. J. Nutrition* **10,** *101 (1956)*). Scurvy, it should be added, occurs in older Bantu children and adults; the condition, therefore, is well recognized. Here then is a context in which a deficiency disease would be expected to be common, at least in urban areas, yet is inexplicably absent.

"Alcoholic" Cirrhosis in the South African Bantu

Although the traditional fermented cereal beverages of the Bantu are low in alcohol concentration, the very large volumes imbibed by a portion of the population (especially in urban areas) result in the ingestion of a level of alcohol per diem probably exceeding the mean for the *drinking* population of the United States (A. R. P. Walker

and Andersson, *Lancet* **2,** *1300* (*1955*)). Nevertheless, "alcoholic" cirrhosis is almost unknown among the Bantu (J. Higginson, *Brit. J. Cancer* **10,** *609* (*1956*)). It may also be pointed out that the Cape "Colored" population (Malay-Hottentot-Caucasian admixture) are, speaking generally, malnourished in comparison with the white population. The former are well known for their partiality for alcoholic drinks. Yet, from necropsy studies, the incidence of cirrhosis among the Cape "Coloreds" (1.66 per cent) is much less than among the white population (5.2 per cent) (J. G. Thomson, *South African Med. J.* **33,** *474* (*1959*)). Although the Cape "Colored" population would be expected to have a high incidence of cirrhosis, they do not.

Myocardial Infarction in the Bantu

Of the ten million South African Bantu nearly one half million are over 60. Economic prosperity in a portion of the population is evidenced by the fact that 100,000 own cars. That there is a measure of Westernization of diet and manner of life is indicated by occasional subjects with high serum cholesterol level, a long blood clot lysis time, and relatively severe arterial lesions. Hypertension is common. Among the moiety who, at least superficially, are Westernized, myocardial infarction would not be expected to be uncommon. Yet, many post-mortem examinations in several centers (B. J. P. Becker, *South African J. Med. Sci.* **11,** *97* (*1946*); Higginson and W. J. Pepler, *J. Clin. Invest.* **33,** *1366* (*1954*); W. Laurie and J. D. Woods, *Lancet* **1,** *231* (*1958*)), combined with extensive electrocardiograph studies carried out on populations at random, reveal myocardial infarction to be virtually absent.

GROUP II

"Nutritional" or Cryptogenic Heart Disease in the Bantu

"Nutritional" heart disease was first described in the South African Bantu in 1951; workers correlated it initially with a poor diet based almost entirely on cereals. Patients improved on ward diet, but the improvement could be reversed upon return to the previous poor diet. The disease is manifested, *inter alia*, by congestive failure associated with enlargement of the heart (Higginson *et al.*, *Brit. Heart J.* **14,** *213* (*1952*)). In Johannesburg, at Baragwanath Hospital, the disease now accounts for 37.5 per cent of total heart disease. Later observers, however, doubt the association with diet; in fact, it has been demonstrated that the disease is encountered in all ages, both sexes, affects both urban and rural Bantu, and appears to occur independently of tribal origin, occupation, educational status, diet, and alcohol and tobacco consumption. "The disease still defies explanation" (Symposium: Cryptogenic Heart Disease in the Bantu of South Africa, *South African Med. J.* **34,** *913* (*1960*)). It appears to be largely exclusive to Africa; even there it is patchy in distribution (H. C. Trowell, *Leech* **28,** *81* (*1958*)).

Gynecomastia in the Bantu

Gynecomastia, or enlargement of the male breast, is not uncommon among the Bantu, being present to a variable degree in about one-third of adults examined at necropsy. Increase in breast tissue is considerably less common (Higginson and A. G. Oettle, *J. Nat. Cancer Inst.* **24,** *589* (*1960*)). On rare occasions enlargement, usually bilateral, reaches 10 cm. in diameter, far transcending the magnitude of the maximum grade 3 depicted in the recent monograph on "Gynaecomastia" (P. F. Hall, *Monograph of Fed. Coun. Brit. Med. Assn. in Australia. No. 2* (*1959*)). Careful inquiries, both personal and by correspondence, indicate that the condition is not seen in such magnitude in South Eastern Europe, the Middle East and India, or the Far East; thus, it seems that severe gynecomastia is limited to Africa. While it was formerly regarded as one of the stigmata of malnutrition (J. N. P. Davies

and Trowell, *Tr. Roy. Soc. Trop. Med. Hyg.* **44,** *756 (1951)*), this view is now less certain, for intensive studies have not revealed any unequivocal dietary, biochemical or pathologic features specific for the condition (Walker, *Ann. N. Y. Acad. Sci.* **69,** *989 (1958)*).

Vesical Stone in Indians

Bladder stone occurs with considerable frequency among the children of Delhi and its environs. Thirty years ago, R. McCarrison concluded that calculus in India was a disease of faulty nutrition, particularly vitamin A deficiency (*Brit. Med. J.* **1,** *1009 (1931)*). Yet recent exhaustive studies in nutrition (and other environmental factors), metabolism, and pathology, have not identified any specific etiological factor or factors (V. Ramalingaswami and A. L. Aurora, *Fed. Proc.* **20,** Suppl. No. 7, 317 (*1961*)). In South Eastern Asia, the foci of occurrence are very restricted. As far as is known, there are no such foci in Central or Southern Africa.

COMMENT

This discussion illustrates that while knowledge is far advanced regarding the predictability of numerous diseases caused or modified by nutrition, such knowledge is far from complete. There are certain diseases which should be more common than they are and certain diseases, whose etiology, formerly ascribed to nutrition, remains unknown. Among local workers such conditions or diseases constitute a serious challenge. They present problems of a type, moreover, in which animal experimentation is likely to give meagre or no help.

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METABOLISM OF FLUORIDES

Ingestion, absorption, and excretion of fluorides vary from individual to individual. Currently there is no convincing evidence to indicate potential fluoride toxicity from the ingestion of water fluoridated at the recommended levels in the United States, because of the presence of an adequate safety factor.

The problems associated with determining the optimal quantity of the trace element fluorine for human consumption have been explored in considerable detail. While these problems have many facets, the over-all relationship of the absorption, excretion, and toxicity of fluorides in man is certainly not particularly complex. Nearly all nutritionists agree that adequate supplies of fluorides in the diet during tooth development result in a major reduction in dental caries incidence. The general acceptance of the benefits of water fluoridation was well reviewed recently by J. M. Dunning (*Nutrition Reviews* **18,** *161 (1960)*). It is true that fluorine is a highly reactive element and that consumption of excessive amounts of its salts by man or by laboratory animals causes serious sequelae. In general there is agreement as to how much fluorine produces toxicity and what the characteristics are of the syndrome of fluorosis. Studies in experimental animals have shown that the renal changes may be related to lower concentrations of fatty acid oxidase (*Ibid.* **18,** *79 (1960)*).

Last year a symposium was published entitled "The Physiologic and Hygienic Aspects of the Absorption of Inorganic Fluorides" (*Arch. Indust. Health* **21,** *303 (1960)*). This symposium included some very interesting observations on various aspects of fluoride metabolism, some of which were reviewed recently (*Nutrition*

Reviews **19,** *198* (*1961*)). A particularly thought-provoking hypothesis raised in this symposium was: "Disadvantageous effects on the bone structure of an adult population may be associated with the prolonged use of drinking water that contains an insufficient concentration of fluoride."

In a recent article G. L. Waldbott (*Arch. Environ. Health* **2,** *155* (*1961*)) commented on this symposium and presented a lengthy series of references about fluoride toxicosis. In essence he challenges the interpretation of certain data in the symposium. In the light of his interpretations, he believes that the amounts of fluoride available from water fluoridation at recommended levels are toxic for a fraction of the population. Thus, his conclusions are at variance with those in the symposium, which are the generally accepted ones in the scientific community. There are essentially no data presented in Waldbott's article that have not already been used widely by opponents to fluoridation of public water supplies.

Waldbott believes that three major shortcomings have been common to most investigations on fluoride metabolism: 1) a great variability of intake, absorption, and excretion of fluorides from one person to another might mean that detailed studies on a limited number of subjects did not necessarily apply to the entire population; 2) relatively few clinical data are available on how fluorides affect man; 3) fluorine is distributed widely throughout the body and is not confined to bones and teeth.

In discussing the symposium in connection with the subject of variations in fluoride intake from one individual to the next, Waldbott listed numerous examples, such as the following ones, from the literature to point out the potential variability in intake. Tables of food content of fluorine indicate wide variations in fluoride content within individual foodstuffs. For example, the fluoride content of mackerel was stated by one author to range from 0.02 p.p.m. to 84.47 p.p.m. Admittedly, the lower value is difficult to explain other than on the basis of a technical error, for this low level is not expected in any plant or animal tissue. The high level is undoubtedly due to the inclusion of a high percentage of bone in the sample analyzed, for normal muscle meat from animals or fish never contains levels of this order of magnitude.

Boiling food in water containing fluoride may increase the fluoride content of the food significantly. Certain insecticides contain fluorides which may remain on the food which has been sprayed. Some of these fluoride-containing chemicals are organic chemicals in which the fluoride is not available. The author also points out that individual food habits are important determinants of the level of fluoride consumed, for example, whether or not an individual drinks tea, for tea contains some fluorides and may be made with water which contains fluorides. Air ordinarily contains very minute amounts of fluoride. In some parts of the world the levels in the air are greatly increased by contamination from factories, resulting in high fluoride concentrations on the surfaces of the leafy vegetables grown nearby and of milk from neighboring cows which have consumed herbage in these areas. Individuals or cattle in these areas undoubtedly absorb some fluorides as a result of inhalation of the contaminated air.

Waldbott points out that the rate of absorption of fluorides varies widely depending upon the form in which the fluorine is consumed. Fluorine in organic compounds is not released in the body since these compounds do not ionize to release the fluoride ion. Therefore, fluorine consumed in organic insecticides or in drugs is not metabolized by the body as fluoride ion and does not contribute to the fluoride pool of the body. Inorganic salts of fluorine may be absorbed less efficiently when consumed along with food than when simply dissolved in water. Numerous laboratory trials have indicated that increases in dietary fat enhance fluoride

retention and the onset and severity of toxicity.

The author mentions that the rate of intake may also be important. Continuous ingestion of the element results in greater storage than does intermittent dosage with the same quantity. Hard water containing high levels of calcium, magnesium, and phosphates retards the rate of fluoride absorption somewhat.

In the course of a short paragraph, Waldbott records the fact that in a clinical study of 77 cases in which 6.8 mg. of fluoride were given as sodium fluoride, as little as 3.6 or as much as 99.5 per cent was recovered in the urine in 24 hours. These data are not even closely similar to excretion data from other investigators and it would be interesting to know more about his procedures and experimental subjects. No comments were made about the total fluoride consumption of these patients from sources other than the test dose.

Waldbott believes that relatively little is known regarding the toxicity of fluorides in man. Osteosclerosis may occur when more than 5 mg. per liter (or 5 p.p.m. of fluoride) is present in the urine of a patient. Crippling fluorosis may occur when fluorides exceed 20 to 80 mg. per liter of fluid. According to these data, drinking water would have to contain approximately 20 to 30 mg. per liter in order to induce clinical illness. However, the author accumulated data from 22 separate reports which occurred in localities where the fluoride content of the water was less than 16 p.p.m. and in most instances less than 8 p.p.m. It is probably noteworthy that amounts of water consumed, the amounts of fluoride from non-water sources, and the influences of malnutrition, high environmental temperatures, and the like, were not considered by the author as he makes this assertion. Obviously the total amount of fluoride consumed is of greater importance than the strict parts per million of fluoride in the drinking water.

He suggested that in any given case the degree of disability due to fluorides is most difficult to assess and that most reports overemphasize biochemical data and most authors have made little or no reference to the accumulation of fluorides in soft tissues. The only definite characteristics of fluoride intoxication are characteristic bone changes as seen by x-ray and characteristic mottling of teeth.

Waldbott believes that fluoride intoxication may cause arthritic pains, joint stiffness, gastrointestinal disturbances, faulty sight and hearing, parasthesias, paraplegias, convulsions, hypochromic normocytic anemia, splenomegaly, and the like. The author concluded that organs were involved in the following order: upper gastrointestinal tract, kidneys, buccal mucosa, and liver. The way in which he has obtained many of these symptoms from questionnaires with leading questions has been described elsewhere (Hornung, *J. Am. Dent. Assn.* **53,** *325 (1956)*). Waldbott believes that there is serious import to the fact that fluorides concentrate selectively in the diseased soft tissues where calcification is taking place. Abnormally high concentrations have been found in cataracts, atheromatous aortas, diseased kidneys, and even in neoplasms. He believes that it is possible, if not probable, that these high concentrations of fluoride may have an effect on the disease process. In general, storage of fluorides in aortas occurs in rough proportion to the degree of aortic sclerosis even in regions where the fluoride content of the water is not high. The author emphasized that no conclusions can be drawn from these data, partially because of the wide variations in intake, the lack of adequate clinical observations, and the inability at the present time to evaluate the effects of fluoride on various organs.

From Waldbott's discussion of the earlier symposium, one might conclude that nothing but confusion existed in connection with the metabolism of fluorides by man. While it

seemed apparent that some of the investigations reviewed and commented upon regarding fluoride metabolism have lacked not quality but depth, many of the investigations upon which our confidence in the safety of water fluoridation is based are substantial studies worthy of our trust.

The fact that fluorides are related primarily to skeletal metabolism may have distracted investigators from the possibility that this element also may have an important role in the metabolism of other tissues. However, the lack of evidence today of soft tissue pathology at recommended levels for human ingestion seems to indicate that there is no need for concern. Possibly, instead of being concerned about the minute amounts of fluoride in all soft tissues as potential hazards, the evidence may come that fluoride ion in appropriate amounts is an important regulator of enzymatic function in the maintenance of homeostasis to prevent excess activity of certain fluoride-sensitive enzymes. The fact that fluoride increases in tissues where calcification is occurring, such as in aortic sclerosis, is to be expected rather than otherwise. Why should calcification occur anywhere in the body without the incorporation of an amount of fluoride commensurate with what is available in the circulatory system?

REDUCTION OF SERUM CHOLESTEROL CONCENTRATION BY TRIPARANOL

Triparanol, an inhibitor of cholesterol biosynthesis, causes a reduction in serum cholesterol concentration in human subjects.

Cholesterol appears sufficiently implicated in the etiology of atherosclerosis that much effort is being devoted to discovering the factors which affect its concentration in the circulating blood. Numerous reviews in this journal have covered the nutritional and endocrine factors which are known to be involved.

In an attempt to find agents which will lower the level of serum cholesterol, investigators have become interested in various other compounds, *e. g.* thyroxine analogues (*Nutrition Reviews* **18,** *249* (*1960*); **19,** *209* (*1961*)) and synthetic drugs which might interfere with cholesterol synthesis (*Ibid.* **19,** *251* (*1961*)). A number of compounds of this class are appearing for clinical trial.

One of these is triparanol ("MER-29"), a product of pharmaceutic research on which an early symposium has been published (*Progress in Cardiovascular Diseases* **2,** *485 1960*)). This drug apparently acts at a late stage of cholesterol synthesis, by inhibiting a single enzymatic step in the transformation of desmosterol to cholesterol. Two recent clinical studies are reviewed below.

P. Lisan, W. Oaks, and J. H. Moyer studied 45 patients with arteriosclerotic heart disease and serum cholesterol levels above 250 mg. per 100 ml. (*Am. J. Cardiol.* **6,** *246* (*1960*)). The subjects, on several triparanol dosage regimens plus their normal diet, were followed up to eight months. Decreases in cholesterol levels of 75 to 100 mg. per 100 ml. were obtained after three months on doses of 250 mg. per day, sooner with 500 or 1,000 mg. The control cholesterol levels of the groups averaged 324 mg. per 100 ml., with standard deviations as high as 66 mg. per 100 ml. In half the subjects, the final serum cholesterol values were lower than 75 mg. per 100 ml. below pretreatment values.

Two patients developed rashes; three (without cirrhosis) had increased bromsulphalein retention, but their pretreatment values were not known.

The authors found that the reduction in serum cholesterol was greatest in those subjects who had the highest levels before treatment. They concluded that a suitable regimen would start treatment with doses of 1,000 or 500 mg. of drug per day, decreasing to a maintenance dose of 250 mg. per day after reduction in the serum cholesterol concentration. When the drug was discontinued, the levels climbed slowly toward control values.

Lisan, Oaks, and Moyer point out that evidence of inhibition of atherogenesis would be very difficult to elicit in man and would require years to obtain. For this reason the symptomatic improvement of the patients with angina is not significant.

The principal difficulties in connection with the work are pointed out by the authors themselves. First, the colorimetric method for cholesterol determination also develops a weaker and interfering color in the presence of 24 - dehydrocholesterol (desmosterol). Since the authors state that 50 to 60 per cent of total blood sterol after triparanol therapy is 24-dehydrocholesterol, this affects the results substantially. Second, the pathologic effects of an accumulation of a near precursor of cholesterol are not known and may well be similar to those of cholesterol itself.

Another investigator, A. Ruskin, has reported the effect of triparanol on the serum cholesterol levels of 30 human subjects (*Arch. Int. Med.* **106,** *803 (1960)*). The patients were 16 to 71 years old and were studied for four to ten months. The diagnoses included hypertension, arteriosclerosis, decompensated cirrhosis of the liver, diabetes, psychoneurosis, nephrotic syndrome, and malabsorption syndrome. Fasting serum cholesterol was measured weekly by the Bloor-Knudson method. A placebo was given during the control period of three or more weeks before the drug was given, generally in doses of 250 mg. per day.

Serum cholesterol values fell in 26 of 30 patients. The average in 24 patients without liver damage fell from 278 to 237 mg. per 100 ml., or 14.7 per cent. Serum cholesterol esters fell from 164 to 151 mg. per 100 ml., or 8 per cent. The decrease occurred in two or three weeks; minimum values were "often delayed up to 12 weeks." Of the six patients with decompensated cirrhosis, two showed no reduction in serum cholesterol. The average for the six fell only from 159 to 147 mg. per 100 ml. (7.5 per cent) and that of the esters from 86 to 83 mg. per 100 ml. However, Ruskin states that the fall in cholesterol level was independent of the control values. In some cases the cholesterol levels began to rise. A larger dose of drug would then reverse this trend: 750 to 2,000 mg. of triparanol per day were required to produce lowering of cholesterol levels in the cirrhotic patients. The cholesterol values returned approximately to normal within one to three weeks after the drug was replaced by a placebo.

There was one unexplained failure of the drug in a 59 year old psychoneurotic Negro woman. Her control cholesterol level was also the lowest, 167 mg. per 100 ml., of any of the patients with normal liver function. In none of the six patients with decompensated cirrhosis of the liver did tests of liver function show any worsening, even on doses of 1,000 mg. of triparanol per day and in some cases more (up to 2,000 mg. per day). Neither did the patients without liver disease show any abnormalities of liver function as appraised by a battery of tests (not including serum alkaline phosphatase) performed weekly for four to ten months. The side effects noted were some euphoria, occasional nausea or oily taste, and infrequently, some fall of blood pressure in patients with hypertension.

Ruskin points out the disadvantages of other regimens and agents for the reduction of serum cholesterol. He indicates the importance of proving safety "from possible deposition in the arterial intima of late precursors of cholesterol, *i. e.* desmosterol, which might accumulate" with triparanol

administration. He realizes that triparanol would have to be given for years before it could be determined whether desmosterol accumulation is innocuous. Ruskin concludes that triparanol is effective in lowering serum cholesterol levels over periods of months and is free from serious toxic effects.

The increasing number of laboratory and clinical reports on drugs which inhibit cholesterol synthesis point up many deficiencies in our knowledge. For one thing, it is not always remembered that the biosynthesis of cholesterol and the control of serum levels are regulated by quite different and perhaps entirely independent mechanisms. The same is presumably true of the accumulation of cholesterol and other lipids in the liver. Species differences and hormonal interrelations remain to be explored. Why, for example, does triparanol not reduce the serum cholesterol level in the capon? It is tacitly assumed that by inhibition of cholesterol synthesis the body can be depleted of cholesterol, and that this in itself is important in preventing or arresting atherosclerosis. The possible atherogenic effects of desmosterol and other earlier precursors have been alluded to and are largely unexplained.

Furthermore, any inhibitor of cholesterol synthesis may well affect the synthesis of various steroids. Indeed, J. C. Melby, M. St. Cyr, and S. L. Dale (*New Engl. J. Med.* **264,** *583 (1961)*) have shown that triparanol reduces the production of cortisol and aldosterone in normal human subjects and impairs the adrenal secretory response to ACTH stimulation and pyrogen stress. These effects were most striking in two patients with hyperadrenalism. Triparanol was extracted in gram amounts from the adrenal tumor removed from one of these patients.

In another paper, R. W. P. Achor, R. K. Winkelmann, and H. O. Perry report on "Cutaneous Side Effects from Use of Triparanol (MER-29): Preliminary Data on Ichthyosis and Loss of Hair" (*Proc. Staff Meetings Mayo Clinic* **36,** *217 (1961)*). Of their seven patients, the two men developed ichthyosis, alopecia, and loss of hair color, documented by photographs in the paper. The five women had pronounced loss of scalp hair; two of them lost some body hair. All the abnormalities were reversible. The authors speculate that the lesions may be due to local interference with cholesterol synthesis, of which the epidermis is an important site.

Thus, the side effects of triparanol are important enough to make it far from innocuous as a therapeutic agent. The criticism is a potential one for any inhibitor of cholesterol synthesis. These agents, however, have proved valuable investigative tools in identifying the metabolic pathways of synthesis. Their clinical use may well yield exceedingly useful information on steroid metabolism and on the mechanisms of the various disparate forms of hypercholesterolemia. Their general therapeutic value, however, is not established and may never be.

PHENYLKETONURIA

The histories of 98 patients with phenylketonuria were reviewed. Victims may have associated inherited defects. Early diagnosis is simple and important, as dietary restriction of phenylalanine may result in correction of the abnormality.

Among the several inborn errors of metabolism, phenylketonuria or phenylpyruvic oligophrenia is singular in that it demonstrates that the lack of a specific enzyme can produce a serious disease resulting in mental incapacitation. Nor-

mally, phenylalanine is converted by phenylalanine hydroxylase to tyrosine, much of which is further changed into melanin. However, individuals who lack this enzyme cannot convert phenylalanine to tyrosine and as a result the concentration of phenylalanine in the blood rises. Some of this is then changed into phenylacetic acid and phenyllactic acid.

If the disorder persists unrecognized in newborn children, irreparable damage to the brain is done by the end of the second year. It may be suspected if a child shows evidence of mental or physical retardation or if the child has convulsive episodes (which occur in about one-fourth of the cases) or if infantile eczema is noted. Most children with this defect tend to have lighter colored skin, hair, and eyes than do their siblings. This coloring may represent a lack of melanin formation. The urine may be noted to have a peculiar musty odor which is due to its content of phenylacetic acid.

Once suspected, the diagnosis is confirmed by one of two simple tests. The addition of ferric chloride to the urine gives a blue-green color; the addition of 2–4 dinitrophenylhydrazine gives a yellow color. A commercial preparation is available in the form of a dip stick known as Phenistix (S. W. Wright, *J. Am. Med. Assn.* **165,** *2079 (1957)*).

Although most clinicians are aware of the existence of phenylketonuria, the condition has been regarded as rare and of only casual interest. Evidence that it is not at all uncommon is given in a report by M. W. Partington (*Canad. Med. Assn. J.* **84,** *985 (1961)*), in which he described 98 patients in the province of Ontario. The diagnosis was established by either the ferric chloride test or the use of Phenistix. It has become customary in many hospitals to put a drop of ferric chloride solution on a wet diaper from each infant during the neonatal period. In most instances this procedure should detect hidden cases, although it is conceivable that some may be missed.

According to most estimates phenylketonuria occurs with a frequency of from two to six cases per 100,000 population. On this basis, assuming an average of four per 100,000, the author estimated that about 240 cases should exist in Ontario. Most of these people eventually go to special schools or mental institutions.

It seems of interest to consider the apparent mortality of patients with phenylketonuria. The author prepared an age-distribution chart which showed that most of the patients were less than 14 years of age and that the highest incidence occurred between the ages of five and nine. Only one patient in his series was over 50 years of age. This type of age distribution is characteristic of mental defectives in general. Comparison of phenylketonuric and other children of the Ontario Hospital School showed a similar distribution. The causes of death of 15 phenyketonuric patients were considered. In eight of them acute respiratory infection was the apparent cause and in another four respiratory infections may have played a role. Two died of measles. Two other deaths were sudden and unexplained. The incidence of childhood eczema was particularly high in those who died.

Children with phenylketonuria may have associated inherited defects. It has been reported that neurofibromatosis (L. S. Penrose, *Lancet* **1,** *572 (1939)*), Gower's muscular dystrophy (S. Coates, A. P. Norman, and L. I. Woolf, *Arch. Dis. Childhood* **32,** *313 (1957)*), and diabetes mellitus (L. S. Penrose, *Am. J. Mental Deficiency* **50,** *4 (1945)*) may be associated with this disorder. Cutaneous disorders are particularly common in these patients. Many of them demonstrate photosensitivity and childhood eczema. In the present series 31 per cent of those in whom an adequate dermatologic history was obtained were found to have eczema, and 16 per cent of the total series had eczema.

Genetic studies are of considerable interest. The disease has been shown to be

autosomal and recessive. The marriage of two heterozygous parents is expected to result in one child with the disorder, two children who are apparently healthy but carry the trait, and one child who is normal. Consanguineous marriages are unusually frequent in the parents of these children. The average incidence of consanguineous marriage in the United States and Canada is estimated at 1 per cent. In the present series consanguineous marriage occurred in 5.8 per cent of the parents. The sex incidence usually shows a slight preponderance of females; in the present series there were 44 males and 54 females. The racial incidence probably is not as important as was originally thought. At first phenylketonuria was considered to be a defect only of Northern European races, but recently it has also been reported throughout Europe, although seldom in Jews. There are scattered reports of its occurrence in other places including Japan, Mexico, and South America. It has affected at least one Brazilian Negro and several other persons of Negroid mixture. In the present series the author had one patient who apparently was a full-blooded Ojibwa Indian. It is of particular interest that she had the usual dark hair, brown eyes, and dark skin of the Indians and did not show any impairment of melanin pigmentation.

Early diagnosis of phenylketonuria is of obvious importance. There is increasing evidence that the early restriction of phenylalanine from the diet of these subjects will result not only in chemical correction of the abnormality, but probably will result in normal mentality. Such restriction has also been reported to decrease the frequency of cutaneous abnormalities and of convulsive disorders. A few patients are first diagnosed as having phenylketonuria after reaching adulthood. Most of these are of extremely limited intelligence, but an occasional patient is encountered who has normal intellect.

This report provides an accurate documentation of an inherited disorder as it occurs among the inhabitants of Ontario, Canada. It is important that physicians become familiar with this syndrome since the adverse consequences are to some extent avoidable if the diagnosis is made early. In addition, the inborn errors of metabolism frequently provide extremely important clues regarding normal metabolic pathways.

BODY COMPOSITION OF INFANTS BORN TO DIABETIC MOTHERS

Infants born to diabetic mothers tend to be heavier and longer than other infants, without exhibiting advanced skeletal maturation. The excess weight of these infants is primarily due to fat.

An appreciable percentage of newborn infants born to diabetic mothers show rather striking abnormalities. As a group, these infants are more frequently affected by congenital anomalies, and have a distinctly higher death rate than normal. They also present a rather characteristic physical appearance. They are large, overweight, plethoric, frequently have hepatosplenomegaly and cardiomegaly, and often suffer from respiratory distress. Hypoglycemia is more commonly encountered than in the normal newborn. This situation has been reviewed in detail by H. C. Miller (*Advances in Pediatrics*, **8**, *137 (1956)*). Of even greater theoretical interest is the fact that these characteristic abnormalities are sometimes seen in infants born to prediabetic mothers, that is, women who do not have diabetes mellitus at the time of delivery, but who are destined to develop the disease in subsequent years.

The clinical picture manifested by these infants is sufficiently characteristic to permit the diagnosis to be made at a glance by the experienced clinician. One question often raised is: do these infants differ from normal in terms of body composition, and if so, how do they differ? For example, is the large size of these infants due to edema of the tissues, or are they merely fat?

An attempt to answer this question has been made recently. M. Osler and J. Pedersen (*Pediatrics* **26,** *985 (1960)*) have applied some of the newer techniques for estimation of body composition to a series of such infants, and have compared the data derived to that obtained with normal babies.

Three groups of newborn infants were studied. Two groups consisted of 122 infants born to diabetic mothers and an equal number (control infants) born at the same gestational age (36 to 38 weeks) to normal mothers. In addition, a number of normal full term infants and normal prematurely born infants were also studied. Certain specialized determinations were made in some of the infants in each group.

Infants born to diabetic mothers were, on the average, 550 g. heavier and 1.5 cm. longer than the control infants. An attempt to assess skeletal maturity was also carried out. The maximum diameters of the ossification centers of the inferior femoral epiphysis and superior tibial epiphysis were measured on roentgenograms. Infants of diabetic mothers and control infants were equal in this respect, though both had smaller epiphysis than the full term infants, presumably because of the shorter gestational period. Thus, despite their large body size, these babies do not exhibit advanced skeletal maturation.

In a smaller number of infants certain other studies were carried out. These included estimation of total body water (deuterium dilution), extracellular fluid volume (thiosulphate dilution), and measurements of thickness of subcutaneous fat (skin-fold calipers or roentgenograms). Measurements of urine volume and nitrogen excretion were also made.

In 23 infants of diabetic mothers, total body water averaged 70.2 per cent of body weight, in contrast to an average of 78.2 per cent for 15 full term infants. In the former group, extracellular fluid volume averaged 38.5 per cent of body weight, in the latter 44.9 per cent. Intracellular fluid volume, calculated by difference, was approximately the same in both groups. Unfortunately, no range of values is given. Such a range would have been of interest in view of the well known variability of such measurements among normal subjects. However, the authors do state that the differences in total body water and extracellular fluid volume are statistically significant.

Since it is established that the fat-free body has a constant water content, the obvious conclusion to be drawn from these data is that the infant born of a diabetic mother has more fat in its body than does the normal infant. This conclusion is supported by measurements of subcutaneous tissue in the leg areas. The full term infants had a thinner layer of subcutaneous tissue (and a relatively larger muscle mass and bone diameter) than did the infants of diabetic mothers. Here, too, the authors give only average values, although they state that the differences are statistically significant.

During the first 24 hours of life, the infants of diabetic mothers had a urine volume three times that of the full term babies, and excreted about two and one-half times as much nitrogen. Excretion of sodium, potassium, and chloride was commensurate with their gestational age. During the second 24 hours of life these differences in urinary excretion diminished markedly.

Determination of total body water was repeated in a few of the infants on the fourth or fifth day of life, when neonatal weight loss was maximum. Six full term infants lost an average of 110 g. in weight and 90 ml. of water during this period.

For the nine babies of diabetic mothers these values were 280 g. and 290 ml. respectively. No data are given, however, on fluid intake during this period.

The authors conclude that the excess weight of these infants is primarily due to fat. Support for this contention can be found in the report of B. Fee and W. B. Weil (*Am. J. Dis. Child.* **100,** *718 (1960)*). These authors chemically analyzed the body of an infant born to a diabetic mother. This infant died during the first day of life. The analysis revealed 21 per cent fat, a somewhat higher value than the 14 to 16 per cent for infants of normal mothers reported in the literature. The water content of the fat-free body was normal. Calculations of body fluid compartments based on electrolyte analyses revealed a slight increase in intracellular water, whereas Osler and Pedersen found no change but rather a decrease in extracellular water.

In discussing the etiology of the changes seen in such infants, Osler and Pedersen conclude that maternal hyperglycemia is the important factor.

More than a decade has passed since the introduction of newer methods, such as dilution techniques, for the estimation of body composition in living man. The authors have put some of these techniques to good use in attempting to elucidate some of the abnormalities which this interesting class of newborn infants exhibits.

ALCHOHOLIC CARDIOMYOPATHY

A clinical syndrome has been reported in middle-aged obese men who regularly drink to excess. The results are myocardial injury and fibrosis.

In recent reviews, alcohol as a factor in causing or preventing heart disease has been discussed. It was reported that alcohol, when added to an atherogenic diet and fed to chickens, resulted in increased concentrations of cholesterol and phospholipids in the serum, but had no apparent effect upon the degree of atherosclerosis of the aortas (*Nutrition Reviews* **18,** *152 (1960)*). Conversely, patients who have alcoholic cirrhosis of the liver were reported to have a decreased frequency of myocardial infarction. Presumably this was related to alterations in the balance between coagulation of blood and fibrinolysis (*Nutrition Reviews* **19,** *37 (1961)*).

W. Evans has described a clinical syndrome which he observed in 80 patients (*Am. Heart J.* **61,** *556 (1961)*). This condition begins insidiously, usually in middle-aged men. Generally the patient is well-nourished or obese and frequently he has abandoned most forms of physical exercise for the gentler art of social drinking. In the cases studied the form of liquor consumed was of the "hard" variety, usually whiskey, gin, rum or brandy. The actual quantity consumed was, of course, difficult to ascertain. However, the author estimated that his patients consumed between six and 12 portions daily for six or more years. Quite obviously the syndrome could occur only in those who were of better than average financial status. It was not observed in beer drinkers, although the latter have been found occasionally to develop beriberi heart disease which the author maintains is quite distinct from the syndrome presented here.

The symptoms began with awareness of palpitation and some breathlessness. Usually the palpitation was caused by extrasystoles and tachycardia or by auricular fibrillation or paroxysmal auricular tachycardia. Although exertional dyspnea was common, anginal pain was not observed frequently. As the disease progressed the patients eventually developed cardiac enlargement

accompanied by both pulmonary and peripheral edema.

Examination of the patient disclosed a rapid heart rate, frequent irregularities as mentioned above, moderate obesity, and usually no evidence of hepatic disease. X-rays of the chest in the early stages showed little change in the size of the heart, but later cardiac dilatation was the rule. Electrocardiograms disclosed changes which the author regarded as "distinctive." The P waves were unaffected and the QRS abnormalities were limited to occasional development of bundle branch block, usually of the right branch, and the presence of a deep T wave in standard leads II and III. However, he regarded the progression of T wave abnormalities as being almost pathognomonic. Early in the disease, when the heart muscle was presumably in a state of increased irritability, the author found tall spiked T waves which he described as "spinous." Later, the T waves became lower or were nearly isoelectric. However, in some leads most of the patients manifested either "cloven" or "dimpled" T waves. In advanced forms of the disease the T waves became inverted, presumably due to myocardial infarction.

In patients who died of the syndrome, autopsy examination disclosed compensatory muscular hypertrophy of the heart, with areas of fibrosis particularly in the region of the Purkinje network. In some instances dense fibrosis was encountered. The author considered the heart to be more vulnerable to fibrosis, and at an earlier date, than is the liver.

The treatment which he advocated consists of complete abstinence from alcohol, a proper diet to provide both adequate nutrition and correction of obesity, and the use of such routine measures as restriction of salt intake, the administration of digitalis, and the use of diuretics when indicated. The response of patients varied considerably depending upon the stage of the disease. Those in whom the diagnosis was made early were observed to respond well. However, once cardiac dilatation and congestive failure occurred the response was less dramatic.

The scientific evidence reported in this article is scanty at best. However, the author referred to his earlier publication (*Brit. Heart J.* **21,** *445 (1959)*), in which he gave greater details on his findings with 20 patients with this syndrome. Apparently this condition is sufficiently distinctive in its clinical features to constitute an entity. The condition occurs without evidence of hepatic disease. It must be distinguished from coronary fibrosis on clinical grounds. Admittedly much criticism might be directed at such dogmatic views; yet it seems to this reviewer that the author has separated a distinct syndrome from the vague and numerous complaints of the obese middle-aged man who drinks too much. The author made no effort to determine what factor or factors resulted in myocardial damage and fibrosis other than the nonspecific "toxic effect" of alcohol. If the occurrence of this syndrome is confirmed by others it would seem fruitful to investigate further the effects of alcohol upon myocardial metabolism.

CALCIUM METABOLISM IN OSTEOPOROSIS

Evidence is presented that osteoporosis in man is the result of a long-term calcium deficiency rather than of decreased osteoblastic activity.

Osteoporosis may be defined as a reduction in the amount of bone without any change in its chemical composition. That is, while the bones are thin they are normal in structure. Recently, B. E. C. Nordin (*Lancet* **1,** *1011 (1961)*) reviewed this field.

In considering the pathogenesis of this disorder it may be assumed, since there is a decreased amount of bone, that there is either a decreased rate of bone formation or an increased rate of bone reabsorption. One view on the pathogenesis of osteoporosis, proposed by F. Albright and E. C. Reifenstein (*The Parathyroid and Metabolic Bone Disease*, Williams and Wilkins, 1948), is that virtually all forms of the disorder are the result of a defect in new bone formation which may be related either to the osteoblasts or to the process of protein synthesis. The latter is necessary to form the bone matrix which may then be calcified. However, since this view was proposed, evidence has been accumulating which requires considerable modification of the original assumptions. Recently, work using isotopic tracers (G. C. H. Bauer, A. Carlsson, and B. Lindquist, *Acta Med. Scandinav.* **158,** *143 (1957)* and Bauer and R. D. Day, *J. Bone Joint Surg.* **40A,** *171 (1958)*) made it possible to measure the bone "accretion rate." Other investigators have noted that patients with osteoporosis did not have a decreased "accretion rate" of bone.

Nordin studied the possibility that increased bone reabsorption was the result of a long-continued negative calcium balance. Since calcium deficiency produces osteoporosis in experimental animals, he studied the role which calcium deficiency may play in the pathogenesis of human osteoporosis. Since direct measurements of the effect of calcium deficiency on the production of osteoporosis in man cannot be made, indirect approaches were employed.

One such approach was to determine the average daily calcium intake in the population of the United Kingdom. The daily calcium intake recommended by the National Research Council of the United States is 800 mg. The British Medical Association Committee on Nutrition suggests a similar amount. Nordin noted that the mean calcium intake for 92 control subjects was 1,200 mg. per day. This amount is similar to that noted by a National Food Survey of the United Kingdom carried out in 1953. The mean value appears quite adequate. However, if one considers the coefficient of variation of the data and assumes a normal distribution of the values, then 5 per cent of the population received less than 500 mg. of calcium per day. Through a series of calculations in which Nordin equated the calcium requirement with the calcium intake, he concluded that 8 per cent of the population has an inadequate calcium intake. In these calculations no allowance is made for the calcium loss through the skin.

Nordin stated that if osteoporosis were due to a calcium deficiency over a long period of time, one might find in patients with osteoporosis evidence of malabsorption of calcium, hypercalcinuria, negative calcium balance or all three. A dietary survey of calcium consumption in 71 patients with osteoporosis and 96 control subjects of similar age and sex was undertaken. None of the patients with osteoporosis had steatorrhea. He found that the mean calcium intake for patients with osteoporosis was 13 ± 1.5 mg. per kg. per day while that for the control was 17.9 ± 1.3. Also, he quotes some unpublished data of R. Smith who studied 71 patients with osteoporosis and 47 normal individuals in the United States. The calcium intake for the patients was 9.9 mg. per kg. per day and that of the controls 12.8. The difference in the calcium intake between normal individuals and patients with osteoporosis was statistically significant with $p < 0.01$.

Nordin also studied the urinary calcium output of patients with osteoporosis and noted that it tended to be higher than that of the control individuals. The calcium excretion was expressed as the ratio of the calcium to creatinine (mg. per mg.). The normal range was found to vary from 0.03 to 0.28. Values in excess of 0.30 were considered indicative of high urinary calcium excretion. In 21 of the 102 patients with

osteoporosis the urinary ratios exceeded 0.30. Nordin found that patients could be divided roughly into two groups, one with a mean excretion ratio of about 0.25 and the other with the mean value at approximately 0.10. The group with a high ratio apparently could not reduce the urine calcium level. The other group was able to reduce the loss of calcium in the urine. However, since all the patients had osteoporosis, they had either a decreased calcium intake or a decreased absorptive capacity. Nordin also reported 40 balance studies with 20 patients with osteoporosis and noted that the urinary calcium level is independent of the calcium intake. However, the urinary phosphorus level was found to vary with the intake of phosphorus. Nordin believes that the patients using renal mechanisms could adapt to a low phosphorus intake but not to a diet low in calcium. The urinary nitrogen level for the same group of patients varied with the nitrogen intake.

Since Nordin believed that patients with osteoporosis were calcium deficient he studied their avidity for administered calcium. When given calcium supplements the patients went into strongly positive balance. From such data he concluded that patients with osteoporosis who did not have steatorrhea did not have a defect in calcium absorption. On this basis he gave supplements of calcium as calcium gluconate (1,100 mg. of calcium per day) to patients with osteoporosis who had complained of back pain for at least one year. Of the 46 patients who received the calcium supplement, 36 were either free of pain or improved clinically following a year of calcium supplementation. No convincing x-ray evidence of recalcification or improvement was noted during this period.

Recently, M. Harrison, R. Fraser, and B. Mullan (*Lancet* **1**, *1015 (1961)*) reported some acute and long-term balance studies in patients with osteoporosis. They assumed that a patient with a calcium deficiency would retain excessive amounts of calcium when given a high calcium intake. Also, the positive balance would probably continue, though at a somewhat reduced level, providing the intake remained adequate until the deficiency was removed. If the patient had osteoporosis due to ineffective osteoblastic activity, one would expect little avidity for calcium when a high calcium intake was supplied. In this series of studies the patients were first given a diet containing a medium amount of calcium (6.7 mg. per kg. per day); later only the calcium content of the diet was increased to a high level (40 mg. per kg. per day). The basal diet remained the same and the excess calcium was given in the form of lactate or gluconate.

On the medium calcium intake (6.7 mg.) all osteoporotic patients appeared to handle calcium in a normal manner. Their net calcium absorption (calcium intake minus fecal calcium) and urinary calcium excretion were normal. These studies, coupled with others carried out by various investigators, indicate no abnormality of calcium metabolism on a medium or low calcium intake in patients with uncomplicated osteoporosis.

When such individuals were switched to a high calcium intake (40 mg. per kg.) changes were noted in the calcium balance. The normal individuals showed a slight increase in calcium lost in the urine immediately after being transferred to the high calcium diet (+1.80 mg. per kg. with a range of 0.54 to 2.9). A moderate increase in net calcium absorption also occurred (average of +5.38 with a range of −0.38 to +9.78 mg. per kg. per day). The patient with osteoporosis had, in general, a slight urinary response to the increased dietary calcium load. The average value for the change in urinary calcium was +0.86 with a range of −0.02 to +2.32 mg. per kg. per day. A much larger net calcium absorption was also noted in the osteoporotic patients (average value +14.02 and the range −1.44 to +38.1 mg per kg. per day). These studies indicate that with the increased dietary

calcium therapy the patients showed greater calcium absorption and perhaps either increased rate of deposition or decreased rate of bone reabsorption. In four of the 12 patients the response to the increased dietary calcium was not different from that of the normal individual.

However, in addition to attaining a more positive calcium balance after receiving a high calcium intake, four of the six patients showed a positive balance after being restudied on the diet for one to three years. Also, the long term calcium therapy seemed to relieve the bone pain.

Both of the groups of studies reviewed indicate that patients with osteoporosis have a marked avidity for calcium. This lends support to the concept that a previous and probably prolonged calcium deficiency rather than a defective osteoblastic mechanism could be the pathogenesis of osteoporosis. With this in mind a rational therapy for such individuals would include a high level of calcium in the diet. Nevertheless, the explanation of calcium deficiency alone may not be sufficient to explain why osteoporosis occurs in only a portion of individuals with a low calcium intake.

MUSCLE COMPOSITION IN KWASHIORKOR

The ionic and metabolic changes of muscle cells which occur in malignant malnutrition were studied in several children who died, and several who recovered, from kwashiorkor.

During the past decade the malignant malnutrition syndrome of infancy and childhood known as kwashiorkor has been the subject of intensive study. Among the many abnormalities manifested by its victims, those having to do with biochemical and physiological disturbances are most intriguing. Many observations have been made on the blood serum of these patients but relatively few on tissue composition. Reports to date indicate depletion of tissue content of potassium, phosphorus, and magnesium (*Nutrition Reviews* **15,** *101 (1957)*; J. C. Waterlow and C. B. Mendes, *Nature* **180,** *1361 (1957)*).

Recently, J. Metcoff *et al.* (*Pediatrics* **26,** *960 (1960)*) studied muscle composition in these patients with regard to both electrolyte content and the concentration of certain metabolites of importance in intermediary carbohydrate metabolism. The hypothesis underlying these studies was that changes in intracellular electrolyte concentration might interfere with one or more enzyme systems, and thus lead to alterations in cell content of certain carbohydrate intermediates.

It is well known that certain electrolytes can modify the activities of a number of enzyme systems (R. W. Von Korff, *J. Biol. Chem.* **203,** *265 (1953)*; J. A. Muntz, *Ann. N. Y. Acad. Sci.* **72,** *415 (1959)*). In general, those ions normally present in high concentration within the cell, such as potassium and magnesium, are enzyme activators. Sodium, on the other hand, exists in low concentration within the cell; if the concentration is raised to levels approximating that of extracellular fluid, a number of enzyme systems are inhibited. To paraphrase A. B. Hastings (*Harvey Lectures* **36,** *66 (1940–41)*), a normal intracellular ionic environment is as important for cell enzyme activity as the extracellular ionic environment is for the activity of the cell as a whole.

As a further test of this hypothesis, the authors compared muscle composition in children who died with that in children who recovered from kwashiorkor.

Eight children suffering from severe kwaskiorkor were studied. A muscle biopsy

was obtained on admission to the hospital, and again seven to ten days later in the survivors and at the time of death in the children who died. Muscle analyses included Na, K, Mg, Cl, fat, water, and non-collagen nitrogen; in addition, analyses of phosphorus (inorganic and organic), pyruvate, phosphoenolpyruvate, isocitrate, alphaketoglutarate, lactate, and oxalacetate contents were made. Comparisons were made with muscle biopsies obtained from well-nourished children.

One fundamental difficulty in studies involving tissue analyses is the choice of a basis of reference. In the realization that most tissue samples contain a variable amount of connective tissue, the composition of which differs considerably from that of muscle cells, some authors have preferred to express data on the basis of non-collagen nitrogen. Metcoff *et al.* express their data in a variety of ways.

On a dry fat-free solids basis, muscle from children with kwashiorkor contained more water and sodium, and less K, P, and Mg than normal. Most of the increase in water content could be accounted for in the extracellular fluid compartment; however, intracellular water was increased at death. The intracellular compartment showed a marked increase in Na concentration and decrease in K and Mg. When expressed in terms of non-collagen nitrogen, K content was not altered, though Mg was significantly decreased.

Accumulation of Na within the cell together with loss of K is a phenomenon common to many types of cell injury—exposure to cold, ischemia, hypoxia, physical trauma. This phenomenon is also seen following exposure of the tissue to certain metabolic inhibitors, and occurs in certain plant forms when deprived of light. It has been observed to occur in many types of cells from a variety of species, and is generally considered to be the reaction of the cell to injury, particularly those injuries which disrupt cell metabolism. Thus, the data of Metcoff *et al.* would lead to the conclusion that the muscle tissues of these patients reflect the consequences of metabolic derangement.

The organic metabolites showed a variety of responses. Muscle pyruvate was decreased while lactate and alphaketoglutarate fell again at death. Values for phosphoenolpyruvate and isocitrate were reduced, while oxalacetate was increased.

In comparing survivors with non-survivors, it was noted that the Na/pyruvate ratio was very high in the latter group; that the phosphorus/pyruvate ratio declined markedly, and that the K/citrate ratio decreased. Other changes were an increase in citrate, Mg, and P relative to alphaketoglutarate. A number of other correlations were attempted without success.

The authors suggest that death in kwashiorkor is associated with a defect in formation of pyruvate from phosphoenolpyruvate in muscle, and some degree of inhibition in the citric acid cycle. They also suggest that the distortion of the intracellular ionic environment is the primary factor responsible for the disruption of intermediary carbohydrate metabolism.

Whether this is in fact the case, or whether the ionic changes are rather a consequence of the metabolic derangement, is a question not easily settled. The theorist has, on the one hand, the information that potassium deficiency and sodium excess can lead to functional failure of the cell; while on the other hand, metabolic derangement is often accompanied by precisely these same alterations in ionic structure. The resolution of this question must await further work, as far as kwashiorkor is concerned.

The detailed observations of Metcoff *et al.* represent a significant step in the study of the problem of malignant malnutrition. It is to be hoped that more studies of cellular function in this serious and all too prevalent condition will be forthcoming.

RELATIONSHIP BETWEEN THYROID HORMONE AND VITAMIN B_{12}

The anemia of myxedema may be explained by impaired absorption of vitamin B_{12}. Rats made hypothyroid by thyroidectomy or propylthiouracil absorbed vitamin B_{12} poorly. This effect was independent of intrinsic factor.

The fact that anemia of a macrocytic type may accompany myxedema was recognized long ago (E. Kraepelin, *Deutsches Arch. f. klin. Med.* **49,** *587 (1892)*). More recent studies of the anemia of myxedema have confirmed the fact that a macrocytic type is more common (S. P. Asper, Jr. and J. G. Wiswell, *Am. J. Med.* **20,** *732 (1956)*), although other forms also may occur. Studies of the bone marrow of patients with myxedematous anemia have shown hypocellularity and fatty replacement (A. R. Axelrod and L. Berman, *Blood* **6,** *436 (1951)*). In a few instances the bone marrow has been shown to contain deposits of hemosiderin (R. C. Douglass and S. D. Jacobson, *J. Clin. Endocrinol.* **17,** *1354 (1957)*).

Since differentiation between myxedema and Addisonian pernicious anemia was difficult for clinicans before elaborate diagnostic methods were available, it is natural that investigators still should be intrigued by evidence which seems to relate the two. In a thoughtful study recently published, evidence is presented which relates absorption of vitamin B_{12} to thyroid hormonal activity (K. Okuda and B. F. Chow, *Endocrinology* **68,** *607 (1961)*). Employing both microbiological measurements of vitamin B_{12} and radioactively tagged vitamin B_{12}, the authors were able to study the concentration of this vitamin in the blood, liver, kidneys, and feces of rats.

A series of studies was devised in order to evaluate the effects of thyroid activity upon absorption, storage, and utilization of vitamin B_{12}. In most of the studies a synthetic diet virtually free of vitamin B_{12} was fed. First they compared normal rats to rats fed desiccated thyroid to determine their respective abilities to absorb a single oral dose of radioactively tagged vitamin B_{12}. They found that in adult female rats the administration of thyroid hormone enhanced intestinal absorption and hepatic concentration, but they were unable to confirm this in adult male rats. Next they compared the effects of feeding the deficient diet to four groups of rats: the first received no treatment, the second received desiccated thyroid, the third received vitamin B_{12}, and the fourth received vitamin B_{12} and desiccated thyroid. The results indicated that the administration of thyroid enhanced absorption of vitamin B_{12} and that these rats stored an increased amount of the vitamin in their kidneys.

Next the authors studied thyroidectomized rats which were fed the deficient diet. Absorption tests with radioactive vitamin B_{12} disclosed that these hypothyroid animals absorbed the vitamin less well than did control animals. Furthermore, this defect could be corrected completely by the administration of either desiccated thyroid or L-thyroxine in appropriate doses. They considered that if thyroid activity could influence the rate of vitamin B_{12} absorption this might be reflected in the concentration of the vitamin in the plasma or liver. Their data did show proportional changes in the concentration of B_{12} in the serum, but strangely enough, thyroidectomized animals had the highest hepatic concentration of the vitamin. Hypothyroidism was induced in another group of rats by feeding thiouracil instead of by thyroidectomy. Again, there was good evidence that the rate of absorption of vitamin B_{12} was impaired.

Next the authors sought to determine whether the effect of thyroid hormone was mediated through the elaboration of intrinsic factor. Accordingly they added intrinsic factor to a single dose of radioactive vitamin B_{12} and injected this directly into

the duodenum of hypothyroid and of euthyroid rats. They found that the rate of absorption in hypothyroid animals was not improved by the addition of intrinsic factor. Carrying their investigation one step further, they employed a special assay technique to determine the quantity of intrinsic factor present in the mucosa of the stomach taken from myxedematous rats as compared with normal rats. They found normal quantities of intrinsic factor to be present.

The authors concluded that thyroid hormone is necessary for gastrointestinal absorption of vitamin B_{12} and that this action is exerted independently of intrinsic factor. Furthermore, they surmised that vitamin B_{12} metabolism is impaired by hypothyroidism, since hypothyroid animals stored more vitamin B_{12} in their liver despite lower blood levels. They were unable to explain the apparent difference in the response of adult female and male rats to desiccated thyroid.

These data might lead one to surmise that the anemia of myxedema is a true vitamin B_{12} deficiency. A peripheral neuropathy has been reported in some patients with myxedema (L. E. Crevasse and R. B. Logue, *Ann. Int. Med.* **50,** *1433 (1959)*). In addition, an effect of vitamin B_{12} upon the tendon reflexes of myxedematous patients has been reported by J. D. Lawson and A. S. Weissbein (*Am. J. Med. Sci.* **239,** *77 (1960)*). While such reports are provocative, the strongest evidence for a vitamin B_{12} deficiency in patients with myxedema would be the finding of megaloblastic arrest in the bone marrow. Careful studies of the bone marrow of such patients have been reported by R. Bomford (*Quart. J. Med* **7,** *495 (1938)*) and by Axelrod and Berman (*loc. cit.*), but there was no evidence of the occurrence of megaloblasts. These reports continue to be of great interest, but the exact interrelationships remain obscure.

THE EFFECT OF LINOLEIC ACID ON DEPOT FAT

The total fatty acid composition of depot fat of rats was analyzed to determine the effect of feeding triglycerides with medium-chain or long-chain fatty acids.

In previous studies it has been demonstrated that rats ingesting long-chain triglycerides have an increased linoleic acid requirement. In contrast, animals fed a fat-free diet or rats fed shorter-chain triglycerides have lower but similar linoleic acid requirements.

The deposition of fat is greater when long-chain triglycerides are fed than when the shorter type is given. As a result of these findings H. Kaunitz, C. A. Slanetz, R. E. Johnson, and V. K. Babayan (*J. Nutrition* **73,** *386 (1961)*) have reported the effect of feeding these two types of triglycerides along with various levels of linoleic acid on the composition of the neutral fat of rats.

The fatty acids of coconut oil and other palm kernel oils were fractionated, analyzed by gas-liquid chromatography, and recombined with glycerol into either medium-chain (MCT) or long-chain triglycerides (LCT). The crude material was alkali-refined, deodorized, and subjected to hydrogen in the presence of a catalyst at high temperature. Following the hydrogenation the material was filtered, decolorized, and refiltered.

The MCT was equivalent to about 15 per cent of the original coconut oil and was a colorless, odorless liquid with a melting point below zero. The LCT was about 60 per cent of the original coconut oil and was a solid fat melting at about 40°C. The iodine numbers were zero in both cases.

The mothers of the male rats used in this study were fed a fat-free diet for four days before the birth of their litters. The young were fed the same diet until the beginning of the experiment, which started at 31 days of age, and ended at 103 days when the animals were sacrificed. The depot fat surrounding the testes was saved for gas chromatographic analysis.

The experimental diets included the original fat-free ration which was modified to contain 20 per cent MCT or LCT at the expense of carbohydrate. In addition to these three diet groups there were five other groups receiving supplements of either 2 per cent or 0.08 or 0.1 per cent added linoleic acid.

The weight gains of the rats fed the MCT diets differed little from the gains of the rats fed the corresponding fat-free diets. The groups fed LCT with little or no added linoleic acid gained less than other corresponding groups. This difference indicated an increased linoleic acid requirement with these triglycerides.

The weight change of testicular depot fat tends to reflect the weight change of total neutral fat, but the composition of depot fat differs in various parts of the body. Testicular fat bodies were heavier in relation to body weight in the groups fed LCT than in the MCT group animals. The composition of the fatty acids from depot fat of animals on the fat-free diet represents the pattern of endogenous fatty acid synthesis. The approximate distribution of fatty acids was 30 per cent palmitate (16–0), 21 per cent palmitoleate (16–1), and 46 per cent oleate (18–1). Marked deposition of linoleate (18–2) was the only striking result of adding 2 per cent linoleate to the fat-free diet. In general, the composition of the nonlinoleate fatty acids was unchanged with the exception of oleate (18–1), which represented 39 per cent of the nonlinoleate part (instead of 46 per cent when the fat free diet was fed).

The MCT consisted of 7.5 per cent caproate (6–0), 78.4 per cent caprylate (8–0), and 14.3 per cent caprate (10–0). Feeding these triglycerides, however, did not produce a different fatty acid pattern of depot fat from that produced with the fat-free diet. In fact, only 1.7 per cent of the fatty acid was C-8 and C-10, with no C-6. Addition of 2 per cent linoleic (18–2) resulted in the deposition of caprylate (8–0), caprate (10–0), and linoleate. The oleate was reduced by about one-half as a result of adding 2 per cent linoleate to the MCT ration. The fatty acid composition of depot fat was influenced more by LCT. Large amounts of lauric (12–0) and myristic (14–0) were deposited and the amounts were increased with the addition of linoleic acid. The fact that only 0.1 per cent linoleic was effective suggested a vitamin-like action.

The composition of depot fat reflects fat intake, synthesis, and degradation. This concept can be amplified on the basis of the present experiments. Linoleate feeding leads to a reduction in neutral fat and promotes the deposition of fatty acids resembling the dietary patterns. In view of the dynamic nature of depot fat tissue, this action of linoleic acid may prove to be very significant.

FATTY ACID ANALYSIS OF LIPID FRACTIONS

Fatty acids from aorta and plasma lipid fractions were compared in normal and cholesterol-fed rabbits.

Lipids accumulate in the aortic intima of cholesterol-fed rabbits. The increase is due either to more synthesis in the aorta or to increased deposition of lipids originally present in plasma. Isotope studies have been useful in providing information regard-

ing the chemical alterations accompanying the development of atheromatous lesions. For example, D. V. Zilversmit, M. L. Shore, and R. F. Ackerman (*Circulation* **9,** *581 (1954)*) reported that various phospholipids of the normal aorta turn over less rapidly than those of the atheromatous aorta. Few of these come from the plasma. In the case of cholesterol, E. Schwenk and D. F. Stevens (*Proc. Soc. Exp. Biol. Med.* **103,** *614 (1960)*) stated that cholesterol turnover is extensive and that a large part of the lesion cholesterol comes from the plasma.

The present study, reported by Zilversmit, C. C. Sweeley, and H. A. I. Newman (*Circulation Research* **9,** *235 (1961)*) was made in order to obtain more information on the origin of lipids in atheroma. This was done by comparing the lipid components of plasma and atheromatous lesions. If the composition of aortic lipid fractions was identical or similar to plasma, this fact could be used as evidence that these lipids came from plasma. On the other hand, differences in lipid composition between aorta and plasma could be interpreted to mean either that the lipids, if deposited from plasma, were altered metabolically or that deposition did not occur.

Albino rabbits were maintained on a stock diet for eight weeks before being divided into two groups. One group was continued on this diet for 16 weeks; the other had added to the diet 1 g. of cholesterol dissolved in 2.6 g. of hydrogenated cottonseed oil. At the end of the experiment, blood, thoracic aorta, and depot fat samples were removed. Lipids were extracted and separated into various fractions using silicic acid with various solvent systems. Fatty acids were determined by gas-liquid chromatography, the values being expressed as weight per cent.

The fatty acid composition of adipose tissue resembled the diet fatty acid composition. Although linoleic acid was present in high concentration in the stock diet, a corresponding increased concentration was not found in adipose tissue.

Consistent differences were not present in fatty acid composition of plasma and arterial triglycerides of cholesterol-fed or control animals. Similarly, triglyceride fatty acid patterns of the media and adventitia did not differ significantly from those in plasma or intima.

Analysis of pooled normal intima was attempted but not enough cholesterol ester was present. The atheromatous intima, however, contained chiefly oleic (18–1), palmitic (16–0), and linoleic (18–2) acids. There was a close similarity between the cholesterol ester fatty acid composition of plasma and intima of cholesterol-fed animals, with the exception that polyunsaturated fatty acids were present in the intima but not in plasma esters.

The analysis of phospholipid distribution showed more choline-containing phospholipids than non-choline types in the atheromatous intima. The lecithin fatty acid patterns of plasma and intima were not affected by cholesterol feeding. Plasma lecithin linoleic (18–2) acid greatly exceeded that of the intima but palmitoleic acid (16–1) was less. The plasma lecithin samples of cholesterol-fed animals contained arachidic (20–0), arachidonic (20–4), behenic (22–0), tricosanoic (23–0), lignoceric (24–0), and nervonic (24–1) acids, but none of these acids was found in the lecithin of intimal lesions.

The sphingomyelin concentration increased in both plasma and aortic intima as a result of cholesterol feeding. Analysis of the fatty acid composition of plasma sphingomyelin showed no great differences between cholesterol-fed and control animals except that palmitoleic acid (16–1) concentration was higher in the controls. Conversely, palmitoleic (16–1) and nervonic (24–1) acids were elevated in the intimal sphingomyelin of cholesterol-fed animals. Lignoceric (24–0) and arachidic (20–0) acid concentrations were less than in the controls.

The sphingomyelin of the intima of control animals had a higher concentration of lignoceric acid (24–0) than plasma. The intimal sphingomyelin of the cholesterol-fed animals compared with plasma had less palmitic (16-0) and more lauric (12-0), myristic (14-0), palmitoleic (16-1), oleic (18-1), and lignoceric (24-0) acids.

In both aorta and plasma and in control and cholesterol-fed animals, the lysolecithin varied from 10 to 20 per cent of the total phospholipid. Fatty acids of lysolecithin from normal intima were characterized by smaller percentages of linoleic acid (18-2) and higher percentages of linolenic acid (18-3) than normal plasma. In the cholesterol-fed animal the lysolecithin of the intima had more lauric (19-0), myristic (14-0), palmitoleic (16-1), and linolenic (18-3) acids and less linoleic acid (18-2) than did plasma.

In an earlier study, Zilversmit, Shore, and Ackerman (*loc. cit.*) reported that there was a rapid turnover of phospholipids of atheromatous lesions of the aorta of cholesterol-fed rabbits. Using P-32 and C-14 acetate, Zilversmit and E. L. McCandless (*J. Lipid Research* **1,** *118 (1959)*) and Newman and Zilversmit (*Circulation* **20,** *967 (1959)*) proposed that the phospholipids are synthesized predominantly in the artery of the rabbit. The present report showing differences in fatty acid composition between plasma and arterial intimal phospholipids helps to substantiate the idea that the phospholipids of the rabbit aorta were not necessarily deposited from the plasma.

There is virtually no contribution of cholesterol synthesized by the aorta to the cholesterol accumulating in the lesion, according to the report of Schwenk and Stevens (*loc. cit.*). With this evidence in mind, it is difficult to interpret the cholesterol ester fatty acid analytical data of the present report in terms of the determination of the site of origin of various esters. One possibility is that there is a selective mechanism which determines the deposition or removal of certain esters. The experiments of this report suggest the possibility that the molecular composition of esterified fatty acids must have been changed extensively if these substances were derived from plasma.

ASCORBIC ACID REQUIREMENT OF GUINEA PIGS

Although an intake of 2 mg. per day of ascorbic acid produced as good growth in guinea pigs as did 8 mg., the reproductive performance of the latter group was superior to that of the former.

No other vitamin besides ascorbic acid has elicited so much debate when the question of its requirement has been raised. Since this vitamin has been clearly recognized for as long or longer than any of the others, it is usually assumed that all problems concerning its quantitative requirement have been carefully worked out. It therefore comes as somewhat of a surprise to learn that "the optimal level of ascorbic acid in the diet of the guinea pig throughout the life cycle has not been definitely established" (O. F. Pye, C. M. Taylor, and P. E. Fontanares, *J. Nutrition* **73,** *236 (1961)*).

The work of Pye and co-workers stems from the hypothesis that "intakes which provide protection from gross signs of scurvy may not be satisfactory for the preservation of optimum health through long periods of time or when the body is subjected to common forms of stress." Although these investigators placed the minimum daily requirement at 0.5 mg. of ascorbic acid for the young guinea pig, they suggest that higher levels produce better health. They

refer to the work of A. F. Abt, S. Von Schuching, and J. H. Roe (*Bull. Johns Hopkins Hosp.* **104,** *163 (1959)*), who found that guinea pigs fed low levels of ascorbic acid remained free from gross symptoms of scurvy. The surgically produced wounds in these animals ruptured at a lower abdominal pressure than did those in the animals receiving a higher level of the vitamin.

Pye and co-workers used as their criteria of adequacy the reproductive performance of female guinea pigs. Four week old guinea pigs were allocated to groups receiving supplements of 2, 4, 6 or 8 mg. of ascorbic acid daily. The ascorbic acid was dissolved in water each day and given by pipette immediately after solution. Two females and one male were placed in each cage. There were 16 to 19 such trios in each of the four groups. A commercial rabbit ration was kept for approximately two to three months to render it "essentially free of ascorbic acid before feeding." Chemical and biological tests were run periodically to verify the absence of ascorbic acid in the feed. Since vitamins other than ascorbic acid may have been destroyed during storage, weekly supplements of vitamins A, D, and alpha-tocopheryl acetate were given throughout the study.

The average weight of the male guinea pig in the four groups was 338 g. at the start of the experiment; that of the females was 326 g. The weight gained during the first year of life was essentially the same for all four groups. Although no data were presented, it was stated that "weight tended to decline in the older pigs, although not quite as much in the 6 and 8 mg. level as in the two lower groups." This reduction may have been associated with the lower fat stores seen in the guinea pigs on the lower levels of ascorbic acid supplementation. A few of the animals fed the 2 mg. of ascorbic acid per day showed slight hemorrhages in the muscles about the knee joints and in the intestinal tract.

In each group some females did not produce young during the first year. For the 2 mg. group this was 35 per cent of the females; for the 4 mg. group it was 13 per cent, and for the 6 and 8 mg. groups it was 23 per cent. These guinea pigs were eliminated from the study.

The reproductive performance of the females receiving 8 mg. of ascorbic acid was superior to that of all other groups. These females had 2.8 pregnancies per productive female, whereas those receiving 6, 4, and 2 mg. of ascorbic acid had 2.1, 2.1, and 1.9 pregnancies respectively.

In the second year of the study approximately 20 per cent of the original females produced young. This was true for each of the supplement groups.

The females receiving 8 mg. of ascorbic acid per day produced a total of 219 young, of which 136 survived, whereas the group receiving 2 mg. produced 90 young with 45 survivors. In both the 4 and 6 mg. groups, the females produced 122 young, of which 69 survived. There was essentially no difference in the young born to each female for the 2, 4, and 6 mg. groups (average of 4.4 young), whereas the females in the 8 mg. group produced one and three-fourths times as many young per female (7.6).

The average birth weight of the young born to the mothers receiving 2 mg. of ascorbic acid was 87 g.; that of the young born to the mothers receiving either 4, 6 or 8 mg. of ascorbic acid was 100 g.

The mortality among the females during the first pregnancy was 36 per cent in the group receiving 2 mg. of ascorbic acid; this dropped to 22, 19, and 17 per cent respectively for the 4, 6, and 8 mg. groups. There was an increase in the average length of the reproductive period for the females from 210 days in the 2 mg. supplemented group to 264 days in the 8 mg. group. There was no significant difference in the life span of the females receiving the different levels of ascorbic acid.

The above study is important in suggesting that there may be a physiological

difference between the minimal amount of a vitamin which will produce good growth and an outwardly healthy state and the "optimal" that will promote maximum efficiency in such functions as reproduction, odontoblast formation, wound healing, and the like. It can only be hoped that confirmatory studies along this line will soon be undertaken so that the evidence can be accepted without reservations.

NICOTINIC ACID AND STARVATION HYPERCHOLESTEREMIA

A 48 hour fast elevated the blood cholesterol levels of rabbits. This effect can be reversed by giving large doses of nicotinic acid.

While nicotinic acid when given in large amounts has been reported to decrease the serum cholesterol levels in both human beings and rabbits, the same effect does not occur when nicotinamide is administered (*Nutrition Reviews* **17,** *78 (1959)*; **17,** *168 (1959)*). The effect of nicotinic acid in lowering the serum cholesterol level is probably not the result of its vitamin activity, but of some pharmacologic effect, since the dosage employed is much greater than that needed to meet the nutritional requirements Also, the amide is not effective.

There have been several explanations offered as to the mechanism of action: some workers believe that there may be an inhibition of cholesterol synthesis; others suggest that there may be some interference in the action of coenzyme A; and some investigators believe that nicotinic acid produces a lowering of serum cholesterol secondarily by inducing anorexia.

Recently, R. Altschul and T. A. Smart (*Metabolism* **10,** *231, (1961)*) have investigated the influence of nicotinic acid on the level of cholesterol in the blood serum of rabbits after a 48 hour fast. It is well known that a diet with a reduced caloric content usually lowers the cholesterol level of the serum. However, there is adequate information in the literature to indicate that during acute starvation there may be an increase in the serum cholesterol level. These investigators used a 48 hour fast as a means of inducing hypercholesteremia in rabbits.

Altschul and Smart studied five groups of rabbits which were first maintained on an adequate diet and then subjected to one of the five following experimental regimens. The first group received no food during the 48 hour experimental period; the second group did not receive either food or water during the experimental period; the third group received a diet which contained only two-thirds of the control caloric intake for the same experimental period; the fourth group received 0.15 g. of nicotinic acid four times a day during the 48 hour fast; and the last group was given the same amount of nicotinic acid per day but maintained on the control diet and not fasted.

Blood samples were obtained from the marginal ear vein for the estimation of the cholesterol level both while the animals were being maintained on the adequate diet and after the 48 hour test period. The group of rabbits which was fasted but allowed water ad libitum during the test period had a mean increase in cholesterol level of the serum of 20.0 mg. per 100 ml. with a standard deviation of ± 22.2 mg. per 100 ml. This increase was statistically significant when compared to the nonfasted condition in the same group of rabbits. There was also an increased serum cholesterol level in the group of animals which was deprived of both food and water for 48 hours (16.05 ± 23.99 mg. per 100 ml.). Again this increase was statistically significant when compared with the period when the animals were fed. However, the difference between the first

and second study groups was not statistically significant (*e.g.* food and water deprivation induced a change in the serum cholesterol level which did not differ from that induced by starvation alone). A third group of animals which was allowed only two-thirds of the regular caloric intake during the 48 hour test period had a significant increase in the serum cholesterol level during the test period as compared with the control period when they were being fed (19.89 ± 17.78 mg. per 100 ml.). This change was not statistically different from the changes induced by either complete food withdrawal or by the effects of fasting and water deprivation. The fourth series of rabbits was fasted and given nicotinic acid during this period. They had a decreased level of cholesterol in their blood serum during the 48 hour fast (average value of 22.4 ± 19.33 mg. per 100 ml.). Thus, the administration of nicotinic acid reversed the effect of starvation on the serum cholesterol levels. Since the animals received no food during the 48 hour test period one cannot attribute the cholesterol lowering effect of nicotinic acid to the result of anorexia. In a fifth series of rabbits it was noted that the nicotinic acid reduced the cholesterol level of the blood even though the animals were being maintained on an adequate diet.

These investigators have shown that the hypercholesteremia induced by a 48 hour fast could be completely reversed by giving the animals large doses of nicotinic acid during the fast. While no explanation is offered for the mechanics of the action of nicotinic acid in reducing the cholesterol levels, these investigators offer strong evidence against the view that nicotinic acid is effective because it induces anorexia. The question arises as to whether the action of high doses of nicotinic acid reported by Altschul and Smart would persist over a period longer than 48 hours and also what effects might be induced on other components of the blood.

ANEMIA, POLYCYTHEMIA, AND LIPID DEPOSITION

The effect of anemia and experimental polycythemia on lipid deposition was studied in hypercholesterolemic rats.

The fact that polycythemia is characterized by an increase in blood viscosity suggests the possibility that this condition may be accompanied by cardiovascular disorders. For example, the slowing of blood flow could conceivably increase the chances of thrombogenesis. However, the search for a correlation between coronary thrombosis and polycythemia is hampered by the small number of patients with coronary artery disease who also have significantly raised hematocrit levels.

Anemia may also be associated with the production of cardiac disease. Cardiac hypertrophy, dilatation, and fatty degeneration may accompany chronic anemia. Although a lipid metabolic disorder may result from severe anemia, no relationship to coronary artery disease has been ascertained. That cardiac hypertrophy may develop during experimental anemia has been known for some time. Rats subjected to simulated high altitude conditions also develop cardiac hypertrophy, according to B. Highman and P. D. Altland (*Arch. Path.* **48,** *503 (1949)*) and are more susceptible to endocarditis (Highman and Altland, *Circulation Research* **3,** *351 (1955)*).

In the present experiment L. C. Fillios, S. B. Andrus, and C. Naito (*J. Applied Physiol.* **16,** *103, 1961*)) studied the effects of anemia, cobalt polycythemia, and high altitude polycythemia in rats on an atherogenic diet.

Three groups of weanling male rats were used. An adequate diet containing 85 per cent skim milk powder with added vitamins and minerals was fed to a group of 16 rats. A second group containing eight rats received enough cobalt chloride to induce polycythemia. A third group received no iron, copper, manganese or cobalt, in order to produce "iron deficiency" anemia. During the 60 days of preliminary feeding the animals were bled periodically to check the extent of polycythemia or anemia.

Control diet rats were divided into two equal groups 11 days before the end of the 60 day period. Of these one group became the high altitude group. They were placed in a low pressure chamber simulating an altitude of 18,000 feet. Animals were returned to sea level pressure for one hour three times weekly in order to facilitate weighing, feeding, and bleeding.

At the end of the 60 day period all diets were supplemented such that each kilogram of diet was mixed with 60 g. of hydrogenated cottonseed oil, 15 g. of cholesterol, and 5 g. of cholic acid. These diets were fed for 90 days. At the end of 90 days the animals were anesthetized and bled. Hearts and aortas were opened and removed, fixed in formalin, sectioned, and stained. Coronary artery sudanophilia, extent of lipid deposition of the aortas, and endocardial involvement were studied.

Growth rates of the anemic and polycythemic groups were comparable to each other but were lower than those of the controls. Hematocrit levels in the control rats were 41 to 50 per cent. During the feeding of the atherogenic diet, the cobalt polycythemia animals' hematocrit levels ranged between 60 and 70 per cent. The anemic rats' levels were between 20 and 29 per cent and the rats undergoing a simulated high altitude averaged 81 per cent.

Serum cholesterol responses differed most between the high altitude polycythemia rats and the cobalt polycythemia animals but the difference was small.

Endocardial sudanophilia was greater in the anemic rats. It was not possible to determine whether the infiltration was due to local tissue anoxia or to circulating cholesterol or to both.

Hearts were weighed after sampling and formalin fixation. The average weight of the high altitude hearts was 1.54 g. The average weight of the hearts from the anemic animals was 1.19 g., which was slightly greater than the 1.11 g. average for the controls and 1.03 g. for the cobalt polycythemia animals.

The differences in coronary lipid accumulation in the high altitude group as compared with the anemia group appeared to be distinct. The mean value of coronary artery sudanophilia for the high altitude rats was 18.1 grid units compared with a value of 6.3 units for the anemia rats. The average number of coronary arteries involved was 1.3 in the anemia group and 3.8 in the high altitude group. None was involved in the other two groups, but due to the small numbers of animals the differences did not appear to be statistically significant.

The endocardial sudanophilia in the two polycythemia groups was similar (10.6 and 9.8 ocular units) but there was no coronary sudanophilia in the cobalt group and 18.1 ocular units in the high altitude group. If the high altitude animals were excluded from consideration, then a correlation did exist between the amounts of endocardial and coronary lipid. In the opinion of the authors, hypoxia could have been involved in the increased coronary lipid of the high altitude group.

No evidence of myocardial infarction was seen either grossly or microscopically. The endocardium contained the most stainable lipid and little lipid was present in the intima of the cornonary arteries or aortas. The lipid present in the arteries was in the media of coronary arteries and aortas often involving the internal elastic membrane. Further experimentation is needed to explore the effects of these variables on the later stages of arterial disease.

COENZYME B_{12} IN LIVER

Coenzyme B_{12} is the major component of the total cobamides of the livers of chicks, rabbits, lambs, and man. However, the mechanism of action of B_{12} coenzymes remains to be solved.

Coenzyme forms of the vitamin B_{12}-group of substances which contain an adenine-sugar moiety in place of cyanide have been isolated from both microbial and animal sources and are of significance in several metabolic processes (*Nutrition Reviews* **19,** *85 (1961)*). In the early work, however, animal organs such as liver were not a preferred source of coenzyme because pronounced losses occurred during extraction and isolation. J. I. Toohey and H. A. Barker (*J. Biol. Chem.* **236,** *560 (1961)*) recently reported the preparation of moderately pure coenzyme B_{12} from the livers of four animal species. They demonstrated that a major portion of the total cobamide in the hot ethanol extract of liver is in the form of dimethyl benzimidazolylcobamide (DBC) coenzyme, now designated as coenzyme B_{12}, and that presumably little or no cyanocobalamin (vitamin B_{12}) occurs as such in liver.

Toohey and Barker used as source material livers from chickens, rabbits, lambs, and man. Because of the relatively small amount of total cobamide in liver compared to that in the microorganisms which were previousy studied, and because of the large amount of lipid in liver, it was necessary to modify somewhat their original isolation procedure (Barker *et al., J. Biol. Chem.* **235,** *480 (1960)*). To facilitate isolation of the coenzyme, Co^{58}-labeled coenzyme B_{12} (DBC coenzyme) was biosynthesized using *Propionibacteria shermanii*. It was isolated in pure form and added to ethanol extracts of liver; distribution of the coenzyme could then be ascertained during fractionation by following the radioactivity. Only very small amounts of the radioactivity, presumably decomposition products of the coenzyme, appeared during liver fractionation in components other than the major coenzyme B_{12} fraction.

The initial steps of the isolation procedure, which consisted of the preparation of an acetone-ethyl ether powder and extraction with hot 80 per cent ethanol, are of importance because they would probably have disrupted a major portion of any protein-bound cobamide. The authors are careful to point out that they are studying the cobamides of liver which are extracted by boiling ethanol and can not therefore distinguish between free and bound forms of coenzyme B_{12}. The ethanol extracts of the livers were subjected to a series of isolation steps. Finally, the extracts were chromatographed on Dowex 50-W from a solution of pH 3 and eluted with sodium acetate buffers of increasing pH. Both light absorbency at 260 mμ (characteristic of cobamides) and radioactivity of the eluted fractions were followed. All steps in the isolation were carried out in very dim light and without exposure to cyanide to minimize decomposition of cobamide coenzymes.

The elution patterns of the liver preparations from each of the four species were similar; however the impurities differed considerably. In each case most of the red color and also nearly all of the radioactivity were eluted together in a position characteristic of cobamide coenzymes containing benzimidazole or its derivatives. Practically no red-colored material occurred in the initial column forerun, indicating an absence or near absence of any cyanocobalamin (vitamin B_{12}); however, small amounts might have been masked by the yellow color of the forerun. Hydroxocobalamin would have adhered to the column and, if present, was not recovered.

The coenzyme B_{12} peak fractions from

each liver preparation were combined and studied with regard to absorption spectra and coenzyme activity in the glutamate isomerase system (Barker *et al., J. Biol. Chem.* **235,** *181 (1960)*). Within the limits of error of the assay all preparations had a coenzyme activity approximately the same as crystalline coenzyme B_{12} (DBC coenzyme) which readily distinguished the liver coenzyme from other known cobamide coenzymes containing benzimidazole derivatives. The benzimidazole base was isolated from the human liver preparation in 93 per cent yield following acid hydrolysis and was clearly identified as 5,6-dimethylbenzimidazole. Because of differential amounts of light-absorbing materials in preparations from the various animal species, the absorption spectra differed greatly. In the purest preparations (human and rabbit livers), however, the spectra resembled that of crystalline coenzyme B_{12} sufficiently to indicate the presence of 5,6-dimethylbenzimidazole as the base component.

The coenzyme B_{12} peak, as measured by *Escherichia coli* bioassay, accounted for 41 per cent (chicken liver) to 73 per cent (human liver) of the total cobamide of the ethanol extracts of liver. Based on the 520 mμ absorbency of the isolated fractions, recoveries of the total cobamide were from 43 to 56 per cent. By making corrections for possible impurities in the coenzyme preparations and for losses of cobamide during isolation, Toohey and Barker "conservatively conclude that at least 48, 53, and 72 per cent of the total cobamide in the ethanol extracts of lamb, human, and chicken livers, respectively, was in the form of coenzyme B_{12}. Since the coenzyme is less stable than the cobamide vitamins, the true percentage of coenzyme in the cobamides of liver may be considerably higher."

This research, which indicates that coenzyme B_{12} is at least a major component of the cobamides of liver, also suggests that cyanocobalamin and hydroxocobalamin, which have previously been isolated from liver, are mainly artifacts of decomposition of the coenzyme by cyanide and by light, respectively. This does not detract from the early work, however, since the isolation of these "artifacts" had a tremendous impact on both fundamental and applied nutrition and biochemistry.

Whether or not the known cobamide coenzymes are the only active forms of the vitamin B_{12} group of substances is certainly not clear at the present time (*Nutrition Reviews, loc. cit*). At least one step in methionine biosynthesis by a mutant of *E. coli* is catalyzed by a vitamin B_{12}-containing protein which is not sensitive to light, as is free coenzyme B_{12}, and is not replaceable by coenzyme B_{12} (R. L. Kisliuk, *J. Biol. Chem.* **236,** *817 (1961)*). The B_{12}-containing group of this protein has not been identified; however, coenzyme B_{12} (DBC coenzyme) is less active than vitamin B_{12} in the formation of the active enzyme (S. Takeyama, F. T. Hatch, and J. M. Buchanan, *J. Biol. Chem.* **236,** *1102 (1961)*). It is possible that coenzyme B_{12} is a decomposition product of this and other enzymes and is not the form of vitamin B_{12} directly involved in synthesis of the holoenzyme.

Although the mechanism of action of B_{12} coenzymes is unknown, the diverse reactions affected by the vitamin are now beginning to show some degree of association. Kisliuk (*loc. cit.*) has suggested that the glutamate isomerase reaction and the succinyl-CoA to methylmalonyl-CoA reaction involve an internal redistribution of electrons, while methyl group formation for incorporation into methionine or thymine and the ribosyl-desoxyribosyl conversion involve a reduction with an external source of electrons. It appears that folacin derivatives may be involved in the second type of reaction but not in the first type. Moreover, A. M. Brownstein and R. H. Abeles (*J. Biol. Chem.* **236,** *1199 (1961)*) have recently demonstrated that in the cobamide

coenzyme dependent conversion of 1,2-propanediol to propionaldehyde, the hydrogen which is transferred from the C-1 position to the C-2 position does not exchange with deuterium in the D_2O solvent system. Thus, vitamin B_{12} coenzymes may be functioning as electron or hydrogen carriers. This is a good working hypothesis which is compatible with the metalloporphyrin-like structure of the vitamin.

PROLONGED PROTEIN DEPLETION

Rats fed a protein-free ration showed a reduction in number of cells per gram of liver and in nitrogen content per cell. Feeding an adequate diet then produced an increase in number of cells, which were also deficient in nitrogen.

Many studies have dealt with experimental animals fed protein-deficient rations for a short time. The biochemical changes resulting from a protracted depletion have been followed in very few of these studies. It is known that the liver shows profound changes during both short and long periods of protein deficiency. As early as 1936, T. D. Addis *et al.* (*J. Biol. Chem.* **116,** *346 (1936)*) showed that when rats are fed a protein-free diet for two days, the liver loses more protein than any other organ. In spite of this loss of protein, R. Elman, M. G. Smith, and L. A. Sachar (*Gastroenterology* **1,** *24 (1943)*) found no change in liver cell size, but did find a loss in stainable cytoplasm.

To extend observations such as these until the animals became moribund, J. N. Williams, Jr. fed adult male rats a purified ration complete in all respects except for the absence of protein (*J. Nutrition* **73,** *199 (1961)*). Control rats were fed the same ration with 20 per cent casein substituted for an equal amount of glucose. The latter rats received the complete ration in an amount equal to that consumed by those receiving the protein-free ration. A third group received the complete ration on an ad libitum basis.

Ninety-six adult male rats were placed on the complete purified ration for ten days to accustom them to the diet. At the end of that time, they weighed 200 g. One-third of the above rats were then placed on the protein-free diet. They lost weight at an exponential rate until the eightieth day, when they weighed approximately 110 g. The pair-fed controls maintained their weight for the first 20 days and then gradually lost weight. By the eightieth day, they weighed approximately 175 g.

Not only was there a difference in the degree of weight lost by the two groups of restricted animals, but the protein-depleted animals developed edema about the eightieth day of the experiment. At that time they had a pouch under the neck which gave the false impression that 4 to 5 ml. of fluid had been injected subcutaneously. The animals receiving a restricted amount of the complete ration showed no signs of edema and did not even appear emaciated.

The nitrogen concentration in the livers of the protein-depleted rats decreased to a value of about 75 per cent by the fortieth day, after which it gradually rose to about 90 per cent. The percentages refer to the values for the ad libitum controls. Beginning with the eightieth day, when the animals were fed unrestricted amounts of the complete ration, there was a rapid decrease in the nitrogen concentration of the livers, which gradually returned to normal by the one hundred and thirtieth day of the experiment. During the first 40 days of the depletion period, the reduction in concentration of liver nitrogen according to Williams is due to an increase in liver glycogen and neutral lipids and a loss of labile nitrogen.

The pair-fed controls showed a gradual

increase in the nitrogen concentration in the liver to a value of approximately 120 per cent of that of the ad libitum controls by the eightieth day. This value dropped to normal within two days of ad libitum feeding. The concentration of nitrogen in the livers was another difference between the protein-depleted and caloric-restricted animals.

The weight of the liver per 100 g. of body weight increased in the protein-depleted rats to a value of 140 per cent by the eightieth day of the experiment. At that time, when the animals were fed a complete ration on an ad libitum basis, the liver increased within a few days to 180 per cent. From that time it gradually decreased to about 120 per cent at the one hundred and thirtieth day of the experiment.

The size of the liver for the pair-fed controls showed a reduction to about 85 per cent by the tenth day of the experiment and remained at that level until the animals were fed on an ad libitum basis. Within two days of unrestricted eating, the liver increased to the same relative size as that in the controls which had been fed on an ad libitum basis throughout the experiment, and remained at that size.

On the basis of deoxyribonucleic acid contents of the liver, Williams calculated that the number of cells in both the protein-depleted and the pair-fed controls showed a gradual reduction to about 65 per cent of the ad libitum fed rats by the fortieth day. The number of cells remained at this level for both groups until they were fed the complete diet on an ad libitum basis. At that time the number of liver cells of the protein-depleted rats was restored to normal within two days; more than 50 days were required for the pair-fed animals. The nitrogen content per cell was maintained at a level of about 50 per cent of that of the ad libitum controls in the livers of the protein-deficient rats after they were fed the complete ration. It appears that the liver regenerated a large number of cells, each with a low nitrogen content, rather than replacing the protein in the existing cells. Similar but less severe changes occurred in the pair-fed controls.

These studies point out that the livers of rats fed a protein-free diet undergo dramatic changes in composition. These changes are different in some respects from those seen in rats fed a restricted amount of a complete ration, as evidenced by the changes in ratio of liver to body weight and the loss of nitrogen per unit of fresh liver. These studies also point out the over-all differences in reaction of animals to these two dietary regimens, as evidenced by the differential weight loss and the development of edema which occurs only in the protein-depleted animals. This work provides a stern warning to the investigator who maintains his animals or subjects on a special diet for a period of approximately one week, at which time he assumes that nutritional equilibrium has been established.

NOTES

The Effect of Insulin on Hepatic Glucose Output in Man

The investigation of the influence of insulin on hepatic glucose metabolism in man has been impeded by the difficulty of measuring hepatic glucose output directly in intact subjects. Numerous workers have employed hepatic vein catheterization in order to study the role of the human liver in glucose metabolism. However, the application of this investigative technique in intact subjects does not permit a distinction between changes occurring in the liver and those arising in the extra-hepatic splanchnic tissues.

It is possible to obtain a direct estimate of hepatic glucose output in patients with end-to-side portacaval venous anastomoses because such an operative procedure separates the hepatic circulation from the blood supply of the remainder of the splanchnic tissues. Such a vascular shunt is produced in some patients with cirrhosis of the liver in an attempt to reduce portal hypertension.

Recently, J. W. Craig, W. R. Drucker, M. Miller, and H. Woodward, Jr. (*Metabolism* **10,** *212 (1961)*) studied the effect of exogenous insulin on the net hepatic glucose output in three patients with cirrhosis of the liver who had end-to-side portacaval venous anastomoses. Two of the individuals also had diabetes mellitus. In these studies, hepatic venous blood was sampled directly from the hepatic vein, using the technique of venous catheterization without general anesthesia. The arterial blood was obtained from the radial artery. In this manner it was possible to measure the glucose levels in blood entering and leaving the liver. The blood flow through the liver was determined by hepatic extraction of administered bromsulfophthalein.

Blood samples for glucose determination were taken at five minute intervals during a 15 minute control period and at five or ten minute intervals during the 50 minutes following insulin administration. Each subject received six to ten units of glucagon-free insulin in a peripheral vein. There appeared to be no consistent change in the estimated hepatic blood flow as a result of insulin administration. Within five or ten minutes after insulin was given, the hepatic venous-arterial blood glucose concentration differences decreased. Before insulin administration the net hepatic glucose outputs were 41.3 and 42.5 mg. per 100 ml. in the two diabetic subjects and 127.8 mg. per 100 ml. in the nondiabetic patient. However, after administration of insulin the values for the diabetic patients were −30.0 and −5.9 mg. per minute while that of the nondiabetic patient was 51.0; the negative values indicate a net uptake of glucose by the liver.

These studies indicate that when insulin is given under the proper conditions there may be not only a reduction in hepatic glucose output but actually a net retention of glucose. In all three patients insulin had a prompt effect in lowering the net hepatic output; in two of the patients a decrease was noted in five minutes, with a definite decrease occurring in all of the individuals ten minutes after the administration of insulin. The promptness of the effect suggests that insulin acts directly on the liver.

However, other investigators (R. Levine and I. B. Fritz, *Diabetes* **5,** *209 (1956)*) have suggested that the effect of insulin may be mediated by an extra-hepatic humoral factor. Whether or not this response of the liver to insulin administration was conditioned by the presence of the portacaval shunt cannot be definitely answered for human beings, since direct measurements of hepatic glucose output have not been made in patients with intact portal circulations.

By utilizing a surgically induced alteration in the blood flow of man, performed to reduce venous hypertension, these investigators have shown that under the conditions of this study the administration of insulin had a prompt effect on the directly measured net hepatic glucose output.

Catalog of Films and Filmstrips on Food and Nutrition

A catalog, "Selected Films and Filmstrips on Food and Nutrition," has been recently published by the Food and Nutrition Council of Greater New York, Inc. This 88 page publication includes brief resumes and appraisals of over 100 films and over 30 filmstrips designed to aid in nutrition programs of various kinds. The catalog was prepared by a standing committee of the Food and Nutrition Council of Greater New York. This volunteer committee includes nutritionists and home economists from various

agencies and educational institutions in the Greater New York area.

In preparing the catalog the committee reviewed over 300 films and filmstrips, selecting those listed as most suitable for educational programs. To assist the person using these materials, the catalog gives information of a general nature on the presentation of films and filmstrips and on the source, length, cost of rental or purchase of each film or filmstrip, indicating the audience for which each is suitable.

Films and filmstrips listed in the catalog are classified according to subject. A wide range of topics relating to food and nutrition is covered, including dental health, career guidance, pregnancy and infant feeding, food preparation, and the food industry. There are interesting films which give an introduction to world food problems. "Food and People" provides a general look at the work of international agencies and a documentary film from India called "The Garden of Gujerat" tells about the development of milk cooperatives in one area of India. A film entitled "People to People," set in Ethiopia, presents the story of United States teams making nutrition surveys in other lands.

Many films deal with such current problems in nutrition education in the United States as food budgeting and weight control. Among the films dealing with food budgeting is one called "Wise Food Buying" which depicts the visit of a middlewestern family to a modern food market. A filmstrip tells about "Ways to Stretch Your Food Dollars."

Among the films dealing with the problem of overweight is a clever animated cartoon on changing food habits entitled "The Owl and Mr. Jones." "Hold That Line" is a film with a rural setting which emphasizes the role of physical activity in weight maintenance. A filmstrip called "The Oopsies" summarizes simple basic facts on weight control. Lecture notes accompany the filmstrip to aid the user in adapting the material to different groups and different situations.

The catalog may be obtained for $1.25 from the Bureau of Publications, Teachers College, Columbia University, New York 27, New York.

Announcement

The Fifth International Food Congress, Inc. has prepared a preliminary announcement concerning plans for the forthcoming Fifth International Food Congress and Exhibition, scheduled to be held at the New York Coliseum, September 8–16, 1962. Hans J. Wolflisberg is Chairman of the organization and Paul S. Willis is Vice Chairman. Further information about the Congress may be obtained from James Muckell, Secretary, Fifth International Food Congress Inc., 527 Madison Avenue, New York 22, N. Y.

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THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

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